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Project Report: Biosustainable Energy and Nutrient Cycles in the Deep Subsurface of Earth and Mars

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Project Progress

Roadmap Objectives

- ***Objective No. 2.1:*** Mars exploration
- ***Objective No. 3.1:*** Sources of prebiotic materials and catalysts
- ***Objective No. 3.2:*** Origins and evolution of functional biomolecules
- ***Objective No. 3.3:*** Origins of energy transduction
- ***Objective No. 3.4:*** Origins of cellularity and protobiological systems
- ***Objective No. 4.3:*** Effects of extraterrestrial events upon the biosphere
- ***Objective No. 5.1:*** Environment–dependent, molecular evolution in microorganisms
- ***Objective No. 5.2:*** Co–evolution of microbial communities
- ***Objective No. 5.3:*** Biochemical adaptation to extreme environments
- ***Objective No. 6.1:*** Environmental changes and the cycling of elements by the biota, communities, and ecosystems
- ***Objective No. 6.2:*** Adaptation and evolution of life beyond Earth
- ***Objective No. 7.2:*** Biosignatures to be sought in nearby planetary systems

Mission Involvement

Mission Class*	***Mission Name (for class 1 or 2) OR Concept (for class 3)***	***Type of Involvement***
3	Mars exploration	Project Investigator

* Mission Class: Select 1 of 3 Mission Class types below to classify your project:

1. Now flying OR Funded & in development (e.g., Mars Odyssey, MER 2003, Kepler)
2. Named mission under study / in development, but not yet funded (e.g., TPF, Mars Lander 2009)
3. Long–lead future mission / societal issues (e.g., far–future Mars or Europa, biomarkers, life definition)

** Type of Involvement = Role / Relationship with Mission
Specify one (or more) of the following: PI, Co–I, Science Team member, planning support, data analysis, background research, instrument/payload development, research or analysis techniques, other (specify).

Principal investigators and collaborators with the IPTAI team are involved in the design and construction of two instruments intended for future exploration of Mars. Tullis Onstott (IPTAI–Princeton) is working with Kevin Lehmann in the Chemistry Department at Princeton on space exploration application for Cavity Ring Down Spectrometry (CRDS). Using a tunable laser, CRDS allows absorption lines for trace gases to be determined with a precision approaching that of isotope mass spectrometry. Consequently, the Princeton group proposes to measure carbon and hydrogen isotopic compositions of Martian methane at ambient atmospheric concentrations (about 1 ppb) using a CRDS instrument mounted on a rover platform. This type of instrument could also be used to look for other trace gases, such as ethane and formaldehyde which are photolytic products of UV breakdown of methane. The sensitivity of CRDM for methane is theoretically sufficient to detect one methanogenic microbe in 10 ml of fluid. As a laboratory proof–of–concept, the Princeton group will determine the isotopic composition of methane produced by a psychrophilic methanogen.

Gary Hieftje in the Chemistry Department at Indiana University is working collaboratively with Lisa Pratt (IPTAI–Indiana) on a life detection probe utilizing a luciferin/luciferace reaction. Luciferin is the basic substrate of all bioluminescence reactions including organisms as diverse as fireflies and marine dinoflagellates. The Indiana luciferin/luciferace probe is based on a reaction that specifically requires ATP as co–factor and is an indicator of bio–available energy or life. A series of initial experiments have been completed and an abstract on this research has been submitted to the Astrobiotechnology Workshop being held at the Carnegie Institution in summer 2004.

Field Expeditions

Field Trip Name: Kinross Lupin Mine

Start Date: 5/10/04	***End Date:*** 5/21/04
Continent: North America	***Country:*** Canada
State/Province: Nunavut Terrotory	***Nearest City/Town:*** Yellowknife
Latitude: 65deg 46min N	***Longitude:*** 111deg 14min W

Name of site(cave, mine, e.g.):	***Keywords:*** microbial, water interaction, permafrost
Kinross Lupin Mine	

Description of Work: Scientists from two NASA Astrobiology Institute teams, IPTAI and MSU, participated in an initial field trip to the Kinross Lupin gold mine in May 2003. Protocols for sampling anaerobic and aerobic eukaryotes will be developed for the Lupin project by a third Astrobiology team which is directed by Mitch Sogin at the Marine Biological Laboratory, Woods Hole Oceanographic Institution. We anticipate scientists from all of these teams participating in a second field trip to Lupin which is tentatively schedule for fall 2004. With the assistance of Timo Ruskeeniemi (Geological Survey of Finland) and Monique Hobbs (Ontario Power Generation), brines of widely varying salinity were collected from 11 subsurface sites in May 2004. Brines below the permafrost were collected from six drill holes outfitted with valves and pressure gages and located at the 1130 and 880 m levels. Dripping water from open fractures in the roof was collected at the 1130 and 250 m levels. Dripping water at the 250 m level is within the current permafrost. Water recirculated within the mine for drilling activities (service water) was sampled from an open drain at the 1130 m level. Samples of water, rocks, biofilms, and microbial mats collected during the May 2004 field trip have been distributed to numerous laboratories and the following research activities are in progress: 1. Growth, isolation and characterization of psychrophilic aerobic and anaerobic microorganisms from environmental (i.e. filter or permafrost cores) samples (Bakermans–Michigan State and Amaral–Marine Biological Laboratory). Characterization of isolates would include phylogenetic identity, membrane lipid composition (Pfiffner–Tennessee), temperature tolerance (including freezing tolerance), salt tolerance, pH range, utilization of substrates (i.e. short chain fatty acids and H2) for carbon and energy sources and stable isotope fractionation (Pratt–Indiana and Sherwood–Lollar–Toronto). 2. DNA (both 16SrDNA and functional genes) analyses of environmental (i.e. filter or permafrost cores) samples and flow cytometry of water samples (Onstott–Princeton) to determine biodiversity and biomass of all microorganisms including those noncultivatable. 3. Phospholipid fatty acid (PLFA) analyses of environmental (i.e. filter or cores) samples (Pfiffner–Tennessee) to determine biodiversity, stress level and biomass of cultivable and noncultivatable. 4. Sulfur isotope analyses of both sulfide and sulfate species (Pratt–Indiana) will be combined with 35S activity measurements and DSR and APS reductase gene analyses to delineate the microbial S cycle in environmental (i.e. both water and rock core) samples. 35S microautoradiography could be performed on freshly obtained rock/permafrost core to determine the spatial distribution of this activity with respect to mineralogy and physical properties. These results of these analyses would also complement the S and O isotopic analyses of sulfate being performed by Frape (Waterloo) to identify the sources of sulfate. 5. Organic chemistry of environmental (water and rock core) samples (Pratt–Indiana) to establish important electron donors and carbon substrates for microbial respiration and growth. The results of these analyses would also complement the inorganic aqueous chemistry being performed by Frape (Waterloo) and Ruskeeniemi (Geological Survey Finland). 6. Dissolved H2 and CO by residual gas analysis water samples (Onstott–Princeton) to establish abundance of these important electron donors for microbial

respiration and growth (CO). These results would also complement the gaseous chemistry analyses being performed by Frape (Waterloo). 7. N−cycle. Concentration and N isotopic composition of dissolved NH4+ (if any) of environmental samples (water and permafrost core) to constrain the origin of N for microbial growth. DNA analyses of functional genes (e.g., NAR, NIR, NOR, NIF, and AMO) utilized by nitrate reducers, N2 fixers and nitrifiers (Onstott−Princeton). These results would also complement the nitrate and N2 isotopic chemistry being performed by Frape (Waterloo). 8. Depending upon the success of 1 and 2, perform microbial activity measurements under in situ conditions and determine the S and C isotopic fractionation associated with microbial sulfate reduction and methanogenesis at low temperatures (Pratt−Indiana, Sherwood−Lollar−Toronto, Bakermans−Michigan State, Pfiffner−Tennessee). These data can be combined with qPCR of the methanogenic gene (MCR) to assess in situ rates of methanogenesis. The results of these studies would complement ongoing characterization of stable isotopic composition of aqueous, gas and rock pore species by Frape (Waterloo). 9. Examine the adaptations of microorganisms to low temperature with depth (which corresponds to time of exposure to low temperatures) to understand the evolution of these low temperature adaptive traits (Bakermans−Michigan State). Examine changes in the composition of the microbial community with depth to determine how low temperatures are selecting for and against individuals within the community. 10. Ground penetrating radar (GPR) survey at low frequencies using the European Space Agency (ESA) Netlander GPR, a version of which is currently deployed around Mars on ESA Mars Odyssey Orbiter. This GPR device is designed to penetrate ~1 km into Martian subsurface, but has not been tested at all on Earth, let alone in a terrain analogous to Mars like that present at Lupin. The subsurface characterization permits unprecedented ground truthing and will constrain interpretations of the current Mars orbiter (Clifford−Universities Space Research Association). An important component of this research project is the development of devices and instruments for borehole analyses, fluid management, life detection, and autonomous drilling in permafrost terrain with low water/rock ratios. Additional funding for instrument development and testing will be sought from NASA by US collaborators and could be sought from the Canadian Space Agency and the Canadian Institute for Advanced Research by Frape and Sherwood−Lollar Another important aspect of coring in permafrost will be the testing of alternative drilling fluids, such as gases, that will minimize contamination, preserve ice and methane clathrates and could be adapted to Martian drilling conditions. Initial time frame for NASA Astrobiology activities at Lupin mine is 3 years.

Members Involved:

Cross Team Collaborations

Scientists from two NASA Astrobiology Institute teams, IPTAI and MSU, participated in a field trip to the Kinross Lupin gold mine in May 2003 for collection of deep subsurface brines from within and below the zone of permafrost.. Protocols for sampling anaerobic and aerobic eukaryotes from

these brines will be developed by Mitch Sogin at the Marine Biological Laboratory. We anticipate scientists from all three of these NAI teams to participate in a second Lupin field trip scheduled tentatively for fall 2004.

Tullis Onstott (IPTAI−Princeton) is working closely with Bruce Jakosky (Colorado) on an agenda for the NAI Subsurface Life Splinter Group. An initial meeting was held in conjunction with the AbSciCon meeting in March 2004 and a second informal meeting of the subsurface group will occur at the MEPEG meeting in July 2004.

Lisa Pratt (IPTAI−Indiana) is a member of the five−person Management Committee for the recently funded (NSF) Field laboratory for Study of Subseafloor Life at the University of Rhode Island. This new facility will strongly enhance NAI activities directed by Steven D Hondt at Rhode Island.